OpenSPIM: A do-it-yourself open access light sheet fluorescence microscope

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Open Access hardware is a relatively recent phenomenon. It pushes the boundaries of innovation by giving anyone the ability to tinker with and improve the original design of scientific instruments or to simply build them and use them for science. One leading example of open access hardware projects is OpenPCR, which allows anyone to build a PCR machine for about 600 dollars. The OpenSPIM project (Fig 1) [1] uses these open access principles to bring light sheet fluorescence microscopy (LSFM) to the broad scientific community.

Light sheet microscopy has been around for over a century. It was initially used for observing Brownian motion of colloids in a liquid medium [2], but only in the last decade has it been coupled with fluorescent labeling and imaging of biological tissues [3]. In LSFM only the focal plane of the detection lens is illuminated by placing a second illumination objective that creates a thin laser light sheet in a perpendicular orientation. This arrangement allows for the use of fast CCD cameras to take a picture of the optical section illuminated by the light sheet and coupled with sample or light sheet movement enables 3D imaging.

Since in LSFM only the part of the specimen that is actually imaged gets illuminated the microscope is very gentle on the samples and allows long-term imaging without significant photodamage and bleaching of the fluorophores. In addition, the LSFM variant Selective Plane Illumination Microscopy (SPIM) can implement sample rotation and thus enable capturing of even large non-transparent samples by imaging from multiple angles.

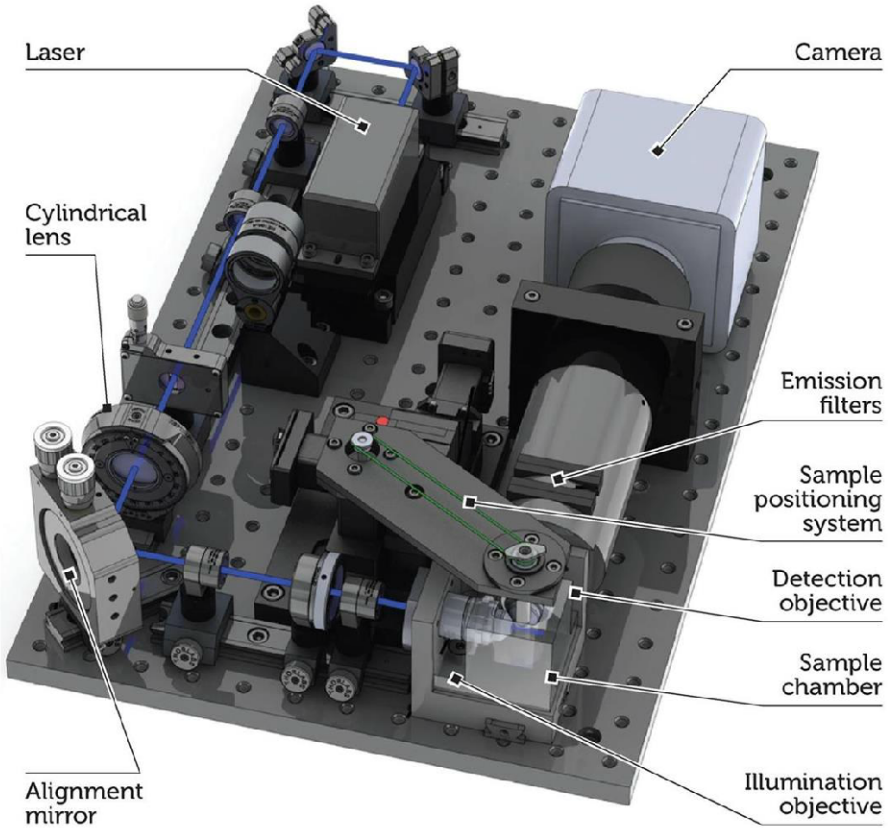

FIGURE 1
Anatomy of an OpenSPIM SolidWorks rendering of an assembled OpenSPIM set-up with crucial components labeled

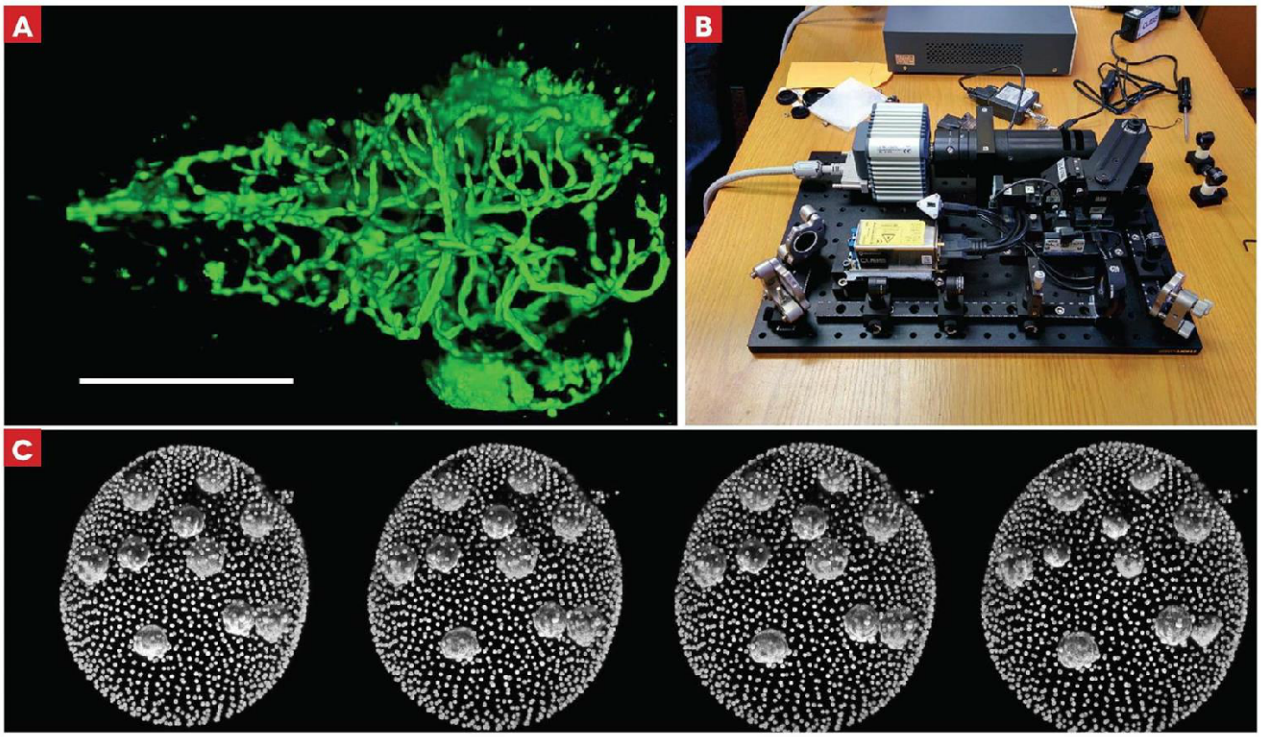

FIGURE 2
Community built OpenSPIM and data
A shows a volumetric rendering of the 4 day post fertilization transgenic (kdrl:GFPla116) zebrafish head with GFP expressed in endothelial cells imaged from 6 different angles acquiring approximately 600µm stacks with a step size of 3µm. The scale bar is equivalent to 300µm. **B** shows an assembled OpenSPIM constructed at the Weinstein lab at the Laboratory of Molecular Genetics in the National Institute of Child Health and Human Development at Bethesda Maryland. **C** shows maximum intensity projection of four time-points from a time lapse of a autofluorescent Volvox specimen imaged at the Department of Applied Mathematics and Theoretical Physics at Cambridge University in England with custom built OpenSPIM

In fact, in light sheet microscopy everything revolves around the sample. In the case of OpenSPIM the sample is suspended vertically in a column of low melting point agarose (typically at 0.5-1.5%) surrounded by an aqueous solution in the focal plane of two horizontally oriented water-dipping objectives that are perpendicular to each other (Fig. 1). This can be achieved in two ways; either the sample is pulled into a glass capillary with a watertight plunger while the agarose is still in liquid form, and then when set it is pushed out into the surrounding medium. Alternatively one can use a type of Teflon™ tubing made out of Fluorinated ethylene propylene (FEP) which has a refractive index similar to water and image the sample through it without significant distortion [4]. Additionally, the rigid medium allows inclusion of sub-resolution fluorescent beads in the agarose, so when it sets they can be used as randomly distributed fiduciary markers for registration purposes.

The vertical orientation of the suspended sample has one major benefit. Since the sample is hanging in front of the horizontally oriented detection lens it can be translated and rotated with a motorized 4-dimensional (x, y, z, & rotation) stage. In that way it is possible to optimally position the sample to collect data from the best angle or to collect information from multiple sides (views) of the sample. This is where the beads mentioned in the previous paragraph can be helpful. Using an open source plug-in in Fiji one can align (register) the views and reconstruct a complete 3D dataset [5].

One can also use the beads to stabilize a sample imaged over extended periods of time [6]. Since the beads are sub-resolution and thus measure the point spread function (PSF) of the microscope, they can also support multi-view deconvolution implemented as another open source Fiji plugin [6]. Deconvolution serves here as a fusion method for combining the data from the different views into a single output image. Additionally, in contrast to other, simpler fusion strategies, deconvolution gives better contrast and increases resolution along the axial direction of the microscope to the cost of relatively demanding computation.

SPIM microscopes have been around since 2004 and the current OpenSPIM set-up is roughly equivalent to the original SPIM implementation developed by Jan Huisken and Ernst Stelzer at EMBL [3]. The key innovation of OpenSPIM platform is that the parts lists and blueprints for the original system are freely available on the OpenSPIM community wiki and that it comes with a complete open source acquisition and analysis suite. Most parts can be purchased and for the custom made ones the wiki offers CAD drawings that can be 3D printed in many cases or made in any mechanical workshop. Moreover the DIY assembly of the microscope is further facilitated by written descriptions, photographs, SolidWorks renderings and videos of the parts and the assembly procedures. The instructions are in fact so detailed that even researchers without prior experience in optical technology development can build an OpenSPIM and it should not take more than one day. It has been said by end users that building OpenSPIM is easier than assembling IKEA furniture or building a Lego model.

The detailed documentation of the OpenSPIM platform extends also to software and the operation of the microscope. The OpenSPIM software builds on the well-established open source acquisition software µManager [7], which enables seamless integration of new hardware components such as cameras or lasers. The µManager OpenSPIM plugin is run inside

FIGURE 3
OpenSPIM as a teaching tool Peter Gabriel Pitrone explaining the principles of light sheet microscopy to African Leadership Academy students during the EMBO practical course on Imaging Infection & Immunity in Pretoria South Africa.

another well-known open source image processing software Fiji [8], where a complete processing pipeline for SPIM data has been developed [9]. The reliance on open source software makes it possible to modify the code to match it with the particular hardware of an OpenSPIM and the biological application at hand.

There are a growing number of commercial LSFM microscopes on the market (Lightsheet Z.1 from Zeiss, Ultramicroscope from Lavision Biotec, iSPIM from Applied Scientific Instrumentation and Ti-diSPIM from Nikon Instruments). All of these systems are very well designed and typically cover a broad range of biological applications from single cells to entire organisms. The ease of use, stability, excellent service and broad range of applications achievable by a single instrument makes the commercial set-ups attractive especially to centralized microscopy facilities. In contrast, the OpenSPIM setup is designed to be adapted to one particular experiment at a time. Additionally, it gives its makers the ability to build an LSFM for the cost of its components and the time it takes to assemble them together.

OpenSPIM was designed originally in Pavel Tomancak's lab, at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden Germany, for observing and cataloging gene expression patterns in developing Drosophila embryos. Due to the sheer amount of genes that need to be imaged, roughly 10,000, a single system would not be sufficient to achieve this goal in a reasonable amount of time. Therefore the initial OpenSPIM prototype was designed optically and computationally to do this one application very well and for relatively modest cost making it possible to build multiple systems (OpenSPIM farms).

At the same time, Pavel Tomancak and his collaborating OpenSPIM development labs Jan Huisken at the MPI_CBG and Kevin Eliceiri, Director of the Laboratory for Optical and Computational Instrumentation (LOCI) at the University of Wisconsin-Madison wanted to build a community around the platform. A community that will take the original, well documented design and run with it, make it useful for their own application, customize it and extend it to a more complex and powerful light sheet paradigms. The open wiki environment was meant to facilitate the open and collaborative design process. It has worked to a certain degree. There is now a growing community of people beyond the original three groups who have built OpenSPIM systems, and the research that the system is used for varies from lab to lab. Here are two examples:

Daniel Castranova, from the Weinstein lab at the Laboratory of Molecular Genetics in the National Institute of Child Health and Human Development at Bethesda Maryland, uses OpenSPIM to observe the formation of blood vessels in the vascular system of developing Zebrafish embryos (Fig. 2a,b [10]). He states: "Building our first OpenSPIM was easier than we had anticipated and the images that we are acquiring with it are better than I had hoped. The video tutorials were very useful and I don't think we would have gone ahead with the project without them. The speed of the system, high dynamic range of the camera, and our ability to rotate the sample and acquire stacks from multiple angles allows us to collect datasets that would be virtually impossible to collect using a traditional confocal for a fraction of the cost."

Drs. Aurelia Honerkamp-Smith and Stephanie Höhn, from the Goldstein Lab at the Department of Applied Mathematics and Theoretical Physics at Cambridge University in England, use OpenSPIM to observe the mechanics of Volvox carteri inversion after the developing embryo finishes its cell divisions using only the native fluorescence of the sample itself (Fig. 2c). Honerkamp-Smith had this to say about OpenSPIM: "The ease of assembly was excellent, I had no trouble at all setting up and getting nice images." and "I think that the project is a huge gift to the scientific community".

The two examples above are great to show what can be done with the existing system as it was originally designed. Yet OpenSPIM is a platform that is easy to modify and continually improve upon. This was done recently at the University of Saint Andrews in Scotland, where researchers incorporated an additional optical component, and tilted another off axis, in the light path of an OpenSPIM system to create an Airy beam light sheet microscope [11].

Based on usage statistics there are altogether more than 50 OpenSPIMs

around the world, however so far very little of that activity reflects back to direct contributions to the OpenSPIM wiki as was intended. It therefore remains a challenge to convince the nascent but growing OpenSPIM community to share its designs with others. The idea of building on others work, extending it and contributing back has taken root in open source software development where widely used licenses (such as GPL) mandate that software derived from open source is kept open. The open access hardware movement is younger and needs to still establish the mechanisms and culture of sharing.

However, there are other examples of OpenSPIM usage that go beyond cutting edge basic research. The original OpenSPIM system design has a footprint that is small enough to fit into a carry-on-luggage container designed for air travel, and has been on multiple international flights. For example, in late March of 2013 the first author of this article took it to the European Molecular Biology Organization's (EMBO) practical course on Imaging Infection & Immunity in Pretoria South Africa. The system was used as a teaching tool for the four groups of course participants who each spent one afternoon to build and image with the system.

Moreover, a class from a prestigious secondary school from the area (African Leadership Academy) visited the course and even these young students were able to build the system in two hours with little assistance (Fig. 3). They completed the construction to such a degree that they were able to see the functioning system and observe a sample.

Thus the low cost and mobility of the OpenSPIM should make it attractive for schools and universities to teach microscopy. The applications for education are multi-faceted as it can cover: optics and the physics of light, additive manufacturing and design, electronics, computer programming, and biology. All of these subjects can be taught together during the assembly and use of an OpenSPIM system. It can even be used in outreach to the general public, since the basic principles of microscopy are exposed in the OpenSPIM set-up much more intuitively than in a conventional microscope.

We are very encouraged that we are not alone in this vision of making light sheet microscopy a experimental playground for open access technology development. Colleagues from Gulbenkian Institute in Lisbon Portugal developed concurrently and independently an OpenSPINMicroscopy microscopy platform that is able to do, next to light sheet, also Optical Projection Tomography [12]. At the recent conference on light sheet microscopy at Centre for Genomic Regulation (CRG) in Barcelona our colleagues unveiled a SPIM system build exclusively using Lego™ parts. Therefore, we look with optimism into the future when the OpenSPIM in its radical openness will demonstrate that the benefits brought to science by the open source approach apply equally well to hardware.

REFERENCES

1 Pitrone PG, Schindelin J, Stuyvenberg L, *et al. OpenSPIM: an open-access light-sheet microscopy platform. Nat Methods.* 2013;10(7):598-9.

2 MLA style: *"Richard Zsigmondy - Nobel Lecture: Properties of Colloids".* Nobelprize.org. Nobel Media AB 2014. Web. 27 Nov 2014. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1925/zsigmondy-lecture.html

3 Huisken J, Swoger J, Del bene F, Wittbrodt J, Stelzer EH. *Optical sectioning deep inside live embryos by selective plane illumination microscopy. Science.* 2004;305(5686):1007-9.

4 Kaufmann, A., Mickoleit, M., Weber, M. & Huisken, J. *Multilayer mounting enables long-term imaging of zebrafish development in a light sheet microscope. Development 139*, 3242-3247 (2012).

5 Preibisch S, Saalfeld S, Schindelin J, Tomancak P. *Software for bead-based registration of selective plane illumination microscopy data. Nat Methods.* 2010;7(6):418-9.

6 Preibisch S, Amat F, Stamataki E, *et al. Efficient Bayesian-based multiview deconvolution. Nat Methods.* 2014;11(6):645-8.

7 Edelstein A, Amodaj N, Hoover K, Vale R, Stuurman N. *Computer control of microscopes using μManager. Curr Protoc Mol Biol.* 2010; Chapter 14: Unit14.20.

8 Schindelin J, Arganda-carreras I, Frise E, et *al. Fiji: an open-source platform for biological-image analysis. Nat Methods.* 2012;9(7):676-82.

9 Schmied C., Stamataki E., Tomancak P. 2014 *Open-source solutions for SPIMage processing. Methods Cell Biol.*, 123, pp. 505-529

10 Choi J, Dong L, Ahn J, Dao D, Hammerschmidt M, Chen JN. *FoxH1 negatively modulates flk1 gene expression and vascular formation in zebrafish. Dev Biol.* 2007;304(2):735-44.

11 Yang Z, Prokopas M, Nylk J, *et al. A compact Airy beam light sheet microscope with a tilted cylindrical lens. Biomed Opt Express.* 2014;5(10):3434-42.

12 Gualda E.J., *et al. OpenSpinMicroscopy: an open-source integrated microscopy platform Nature Methods* 10, 599-600 (2013) doi:10.1038/nmeth.2508

BIOGRAPHY

Peter Gabriel Pitrone works at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden Germany as a Technologist in Dr. Pavel Tomancak's research group. He designs and builds Open Access Light Sheet Fluorescence Microscopes for observing gene expression patterns in developing fruit fly embryos.

ABSTRACT

The OpenSPIM project is a marriage of light sheet fluorescence microscopy (LSFM) and the open access movement. The goal of the project is to bring LSFM to the forefront of the scientific community's toolbox for imaging live specimens in the sub-centimeter range. This article discusses the conceptual basics of LSFM, what the OpenSPIM system is and what it does, and visions of where the project can go in the future. There are a few testimonials and some preliminary data from the system's users, as well as an example of how it can be used as a prototype for further development.

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